Review Article

Cell mediated immunity to fungi: a reassessment

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Protective immunity against fungal pathogens is achieved by the integration of two distinct arms of the immune system, the innate and adaptive responses. Innate and adaptive immune responses are intimately linked and controlled by sets of molecules and receptors that act to generate the most effective form of immunity for protection against fungal pathogens. The decision of how to respond will still be primarily determined by interactions between pathogens and cells of the innate immune system, but the actions of T cells will feed back into this dynamic equilibrium to regulate the balance between tolerogenic and inflammatory responses. In the last two decades, the immunopathogenesis of fungal infections and fungal diseases was explained primarily in terms of Th1/Th2 balance. Although Th1 responses driven by the IL-12/IFN-γ axis are central to protection against fungi, other cytokines and T cell-dependent pathways have come of age. The newly described Th17 developmental pathway may play an inflammatory role previously attributed to uncontrolled Th1 responses and serves to accommodate the seemingly paradoxical association of chronic inflammatory responses with fungal persistence in the face of an ongoing inflammation. Regulatory T cells in their capacity to inhibit aspects of innate and adaptive antifungal immunity have become an integral component of immune resistance to fungi, and provide the host with immune defense mechanisms adequate for protection, without necessarily eliminating fungal pathogens – which would impair immune memory – or causing an unacceptable level of tissue damage. The enzyme indoleamine 2,3-dioxygenase and tryptophan metabolites contribute to immune homeostasis by inducing Tregs and taming overzealous or heightened inflammatory responses.

Keywords fungal infections, Th/Treg subsets, inflammation, immunity

Introduction

Fungal infections and diseases

The kingdom of fungi comprises a number of species that are associated with a wide spectrum of diseases in humans and animals, ranging from allergy and auto-immunity to life-threatening infections [1]. Most fungi (such as Histoplasma capsulatum, Paracoccidioides brasiliensis, Coccidioides immitis, Blastomyces dermatitidis, Cryptococcus neoformans, Aspergillus fumigatus and Pneumocystis jirovecii) are ubiquitous in the environment. Some, including Candida albicans, establish lifelong commensalism on human body surfaces. Opportunistic fungal infections occur in patients with defective immunity. Despite marked reductions in the rates of AIDS-associated fungal infections, such as cryptococcosis in developed countries, the burden of these diseases in developing countries is large and increasing. Cutaneous fungal infections in the oncology patients are also being recognized. Candida species...
remain the fourth most important cause of hospital-acquired bloodstream infections. Invasive aspergillosis, mostly by *Aspergillus fumigatus* and *A. terreus*, and other mould infections are a leading cause of infection-related death in hematopoietic stem cell transplant recipients.

Fungal diseases include type I hypersensitivity, the most prevalent disease caused by airborne fungi, including species of *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium*, and a large number of other illnesses, including allergic bronchopulmonary mycoses, allergic chronic sinusitis, hypersensitivity pneumonitis and atopie eczema/dermatitis syndrome (AEDS; formerly atopic dermatitis) [2]. Sensitization to molds has been reported in patients with asthma and allergic bronchopulmonary aspergillosis (ABPA) is frequent in patients with asthma and cystic fibrosis [3]. There is evidence that fungal sensitization also contributes to autoreactivity against self-antigens due to shared epitopes with homologous fungal allergens [4].

The two most common cutaneous fungal infections are caused by one or more of the fungal species in the keratinophilic genera *Microsporum*, *Trichophyton*, or *Epidermophyton* and *Malassezia* spp. Although *Malassezia* yeasts are a part of the normal microflora, they have been associated with a number of diseases affecting the human skin, such as pityriasis versicolor, folliculitis, seborrheic dermatitis and dandruff, AEDS, psoriasis, and—less commonly—with other dermatologic disorders [5]. Recent studies of the interaction of *Malassezia* spp. with innate cells have highlighted the potential of the fungus to modulate the immune response directed against it. In the normal skin, the fungus may down regulate the inflammatory response, through the production of immunosuppressive transforming growth factor (TGF)-β1 and interleukin (IL)-10, allowing it to live as a commensal. In contrast, in AEDS and psoriasis, both chronic inflammatory skin diseases, the skin barrier may provide an environment that can enhance the release of allergens as well as the ability of the fungus to up-regulate the production of molecules involved in hyperproliferation and cell migration thus exacerbating psoriatic lesions [5]. Therefore, an inflammatory circle seems to be at work, the manipulation of which, may offer strategies to control or prevent exacerbations of these diseases. A similar vicious circle may be at work in chronic mucocutaneous candidiasis (CMC), a primary immune deficiency presenting as an inability to clear yeasts, mostly *C. albicans*, which consequently persist and recur in infections of the skin, nails and mucous membranes [6]. Most CMC patients also develop accompanying endocrine and inflammatory disorders that suggest an underlying deregulation of the inflammatory and immune responses [6].

These findings highlight two major features of mucosal fungal diseases—that is, molecular mimicry as a basic mechanism involved in perpetration of chronic allergic diseases [4,7–9] and the deleterious effect of a deregulated inflammation to which both the host and the fungus contribute [6]. Both features underline the existence of complex and unusual relationships of pathogenic and opportunistic fungi with the vertebrate immune system, partly due to some prominent features. Among these, the ability to reversibly switch from one form to the other in infection which may have resulted in an expanded repertoire of cross-regulatory and overlapping antifungal host responses at different body sites; and, for commensals, the highly effective strategies of immune evasion they must have evolved to survive in the host environment. However, because fungal diseases are rare, a stable host-parasite interaction is a likely condition for most, potentially pathogenic, fungi. This condition requires that the elicited immune response be strong enough to allow host survival with or without pathogen elimination and to establish commensalism/persistence without excessive pro-inflammatory pathology. Therefore, the balance of pro-inflammatory and anti-inflammatory signaling is a prerequisite for successful host/fungal interactions and requires the coordinate actions of both innate and adaptive immune systems [10,11].

The aim of this review is to re-evaluate the role of cell-mediated immunity and inflammation in response to fungi, and to accommodate the recent developments in Th cell subset diversifications and functions within the diversity of fungi and their pathogenicity.

**Inflammation and immunity: from protection to disease promotion**

Inflammation is a key feature of fungal infections and diseases. The inflammatory response may serve to limit infection but an overzealous or heightened inflammatory response may contribute significantly to the histological patterns and pathogenicity, as documented by the occurrence of severe fungal infections in patients with immunoreconstitution disease, an entity characterised by localised and systemic inflammatory reactions of varying degrees that have both beneficial and deleterious effects on infection [12–15]. These patients may experience severe, often intractable, fungal diseases, with variable levels of fungal burden, despite recovery from neutropenia and the occurrence of pathogen-specific immunity. Below are few examples of fungal infections and diseases paradoxically associated with immune activation. For *Candida*, known to resort to a number of strategies to down-regulate
inflammation and favor commensalism [16,17], the failure to resolve inflammation associated with defective fungal clearance characterizes CMC (see below) and hepatosplenic candidiasis (HSC), for which a pathogenetic role of immunoreconstitution has recently been postulated [18]. One most striking paradox in HSC is the inefficacy of antifungal treatment and the beneficial effects of corticosteroid therapy [18]. Moreover, in patients with Candida systemic infection, the clinical course and mortality were predicted by systemic inflammatory response and APACHE II score, but not by the infecting species [19].

For Aspergillus, the association of persistent inflammation with intractable infection is common in non-neutropenic patients after allogeneic hematopoietic stem cell transplantation [20], as well as in allergic fungal diseases [21]. In patients with Chronic Granulomatous Diseases (CGD), both a heightened inflammatory response and defective fungal clearance are observed [22,23]. A systemic inflammatory response syndrome with progressive cardiorespiratory failure has been recently described in patients with C. immitis fungemia [24], and high levels of pro-inflammatory cytokines are associated with disease severity in patients with paracoccidioidomycosis [25]. These findings extend the concept of deleterious inflammation to non opportunistic mycoses.

The inflammatory response is initially mediated by cells of the innate immune system followed by a later adaptive immune response, which responds to the signals originated by the innate immune system [26]. Most of the innate mechanisms are inducible upon infection and their activation requires specific recognition of invariant evolutionarily conserved molecular structures shared by large groups of pathogens by a set of pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) and C-type lectins [26–28]. Innate and adaptive immune responses are intimately linked and controlled by sets of molecules and receptors that act to generate the most effective form of immunity for protection against fungal pathogens [26,29–31]. The decision of how to respond will still be primarily determined by interactions between pathogens and cells of the innate immune system, but the actions of T cells will feed back into this dynamic equilibrium to suppress overzealous innate responses [11]. That conventional adaptive T cells may suppress the early innate immunity has recently been shown [32].

In the last two decades, the immunopathogenesis of fungal infections and associated diseases was explained primarily in terms of Th1/Th2 balance [33,34]. The dichotomous Th-cell model has proven to be a useful construct that sheds light on the general principle that diverse effector functions are required for eradication of different fungal infections. However, while the pathogenetic role of either subset may still hold true, the reciprocal regulation of both subsets is apparently outdated. Although it is currently believed that Th1 responses driven by the IL-12/IFN-γ axis are central to protection against fungi, it is also an undisputed fact that patients with inborn deficits in the IL-12/IL-23-IFN-γ loop do not demonstrate increased susceptibility to fungi [35], with few exceptions [36,37]. This implies that other cytokine pathways may also play a role. In this regard, the new entry, the Th17 pathway, playing an inflammatory role previously attributed to uncontrolled Th1 cell reactivity [11,38,39], has highlighted the molecular connection between the failure to resolve inflammation, lack of antifungal immune resistance and susceptibility to chronic fungal infections and diseases [11].

### From pathogen sensing to immune activation

**The TLR and non-TLR detection system**

Antigen-independent recognition of fungi by the innate immune system leads to the immediate mobilization of immune effector and regulatory mechanisms that provide the host with the rapid initiation of the immune response and creation of the inflammatory and co-stimulatory environment for antigen recognition; establishment of a first line of defense, which holds the pathogen in check during the maturation of the adaptive immune response; and steering of the adaptive immune response towards the cellular or humoral elements that are most appropriate for protection against the specific pathogen. Receptors on phagocytes not only mediate downstream intracellular events related to clearance, but also participate in complex and disparate functions related to immunomodulation and activation of immunity, depending on cell type. Therefore, in order to achieve optimal activation of antigen-specific adaptive immunity, it is first necessary to activate the pathogen-detection mechanisms of the innate immune response.

A number of cell wall components of fungi may act through several distinct PRRs, each activating specific antifungal programmes on phagocytes and dendritic cells (DCs) [26,31,40,41] but cooperating for full immune cell activation [42,43]. The environmental set up probably affects PRR functioning, given that the optimal ability of cells to phagocytose fungi is observed in the environment where a pathogen is naturally encountered [44]. However, another function of the innate immunity that is emerging is that it also has a

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role in sterile inflammation – that is, inflammation caused by endogenous TLR ligands. In this regard, TLR activation itself is a double-edged sword. Thus, members of the TLR family are involved in the pathogenesis of autoimmune, chronic inflammatory disorders such as asthma, rheumatoid arthritis, and infectious diseases. By hyperinduction of pro-inflammatory cytokines, by facilitating tissue damage or by impairing protective immunity, TLRs might also promote the pathogenesis of infections [45]. Not surprisingly, therefore, the exploitation of PRRs may also provide a mechanism to divert and subvert host immune responses by fungi [46]. Table 1 summarizes some major features of TLR recognition of fungi.

C-type lectin receptors (i.e., dectin-1 and 2, DC-SIGN, Mannose Receptor and the galectin family) are the prototype of innate non-TLR signalling pathway for antifungal sensing [28,64]. Dectin-1 is a myeloid-expressed transmembrane receptor, possessing a single extracellular non-classical C-type-lectin-like domain that specifically recognizes the cell wall carbohydrate 1,3-glucans of many fungi [65–74]. As for TLRs, avoiding dectin-1 recognition could be a counterstrategy of fungi for immune evasion [17,69,75]. The cytoplasmic tail of the receptor contains an immunoreceptor tyrosine-based activation-like motif related to those of adaptive antigen receptors which can mediate myeloid cell activation, cytokine production and a variety of anti-fungal responses through the tyrosine kinase Syk/ cytoplasmic caspase-recruiting domain (Card9)-dependent pathways [76,77].

### Table 1  Major features of TLR recognition of fungi.

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Dendritic cells

Dendritic cells (DCs) are uniquely adept at decoding the fungus-associated information and translating it into qualitatively different adaptive T-cell immune responses [78,79]. DCs are capable of internalizing different fungal morphotypes through different receptors and forms of phagocytosis. The ability of a given DC subset to respond with flexible activating programs to the different stimuli as well as the ability of different subsets to convert into each others confer unexpected plasticity to the DC system [79]. The results are consistent with the view that fungi have exploited common pathways for entry into DCs, which may include a lectin-like pathway for unicellular forms and opsono-dependent pathways for filamentous forms [79].

A number of PRRs determine the functional plasticity of DCs in response to fungi and contribute to the discriminative recognition of the different fungal morphotypes. This process exemplifies the importance of PRRs and TLRs not only in direct early immune responses, but also in orchestrating the adaptive immunity [38,39,77,79] (Table 1). Dectin-1 has recently gained attention being involved in Th17 cell activation in response to *Candida* at least *in vitro* [77] (Fig. 1).

Fungus-pulsed DCs translated fungus-associated information to Th1, Th2, Th17 and Treg cells, *in vitro* and *in vivo* upon infusion of different DC subsets [38,79,80]. For instance, the infusion of plasmacytoid DCs in bone marrow-transplanted mice resulted in the concomitant Th1/Treg cell priming eventually leading to fungal growth restriction, limited inflammatory pathology and, interestingly, transplantation tolerance [80]. These results, along with the finding that fungus pulsed DCs could reverse T cell anergy of patients with fungal diseases, may suggest the utility of DCs for fungal vaccines and vaccination [79,81].

By subverting the morphotype-specific program of activation of DCs, innate environmental factors qualitatively affect DC functioning and Th/Treg selection *in vivo*, ultimately impacting on fungal virulence. The model accommodates the concept of virulence as an important component of fungus fitness in vivo within the plasticity of immune responses orchestrated by DCs. The type of cell signaling initiated by the ligand receptor interaction in DCs determines the functional plasticity of DCs at the fungus/host interface and the
qualitative development of disparate Th-cell responses [78,82].

**The adaptive cell-mediated immunity**

Serological and skin reactivity surveys indicate that fungal infections are common but clinical disease is rare, consistent with the development of acquired cell-mediated immunity (CMI). Lymphocytes from healthy subjects show strong proliferative responses after stimulation with fungal antigens and produce a number of different cytokines [26]. For dimorphic fungi, the initial exposure either is asymptomatic or results in mild infection that confers protective immunity. In contrast, the severity of the disease correlates with the degree of the impairment of CMI and elevated levels of antibodies. For *C. neoformans*, the high prevalence of antibodies to cryptococcal antigens in normal individuals suggests that primary infection is followed by fungal growth restriction and concomitant immunity as recently demonstrated in experimental cryptococcosis [83,84]. As a matter of fact, direct inhibition of T-cell proliferation by fungal polysaccharides may underlie the defective CMI of patients with persistent cryptococcal infections [85]. Underlying acquired immunity to *C. albicans*, such as the expression of a positive delayed type hypersensitivity (DTH), is demonstrable in adult immunocompetent individuals.

There is extensive plasticity in the T-cell response to fungi. The heterogeneity of the CD4+ and CD8+ T cell repertoire may account for the multiplicity and redundancy of effector mechanisms through which T lymphocytes participate in the control of fungal infections. These may include direct antifungal activity, apoptosis and complex effector functions resulting from the dynamic interactions between T cells bearing selected members of the Vβ families of the T cell receptor [86–90]. The functional plasticity is such to uncover vaccine potential in conditions of immunodeficiency [91]. The flexible program of the T lymphocytes also implicates the production of a number of mediators, including cytokines. Due to their action on circulating leukocytes, the cytokines produced by fungus-specific T cells are instrumental in mobilizing and activating antifungal effectors, thus

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**Fig. 1** Recognition of *Candida albicans* by dendritic cells. Dendritic cells sample fungi at the site of colonization/infection, transport them to the draining lymph nodes and activate disparate Th/Tregs cells in a morphotype- and tissue-dependent fashion. Fungal recognition by TLRs occur in a morphotype-specific manner and cooperates with curdlan-recognition by dectin-1. This results in the activation of distinct programs in dendritic cells culminating in cytokine-dependent Th cell activation. Those include activation of an immunogenic, MyD88-dependent program culminating in the production of IL-12 – and eventually leading to protective Th1 cells – or, in cooperation with dectin-1, of IL-23, and possibly other inflammatory cytokines, which activate pathogenic Th17 cells – promoting inflammation and dampening Th1 responses. By inducing IL-10, the tolerogenic, TRIF-dependent pathway contributes to regulatory T cell induction – balancing innate and adaptive inflammatory immunity. By an as yet unknown receptor and downstream cellular events, the production of IL-4 leads to activation of Th2 responses opposing antifungal function in effector cells and promoting allergic manifestations. CARD9, cytoplasmic caspase-recruiting domain 9; MyD88, *Drosophila* myeloid differentiation primary response gene 88; TRIF, Toll/IL-1 receptor (TIR)-domain-containing adaptor protein inducing IFN(β); ITAM, immunoreceptor tyrosine-based activation-like motif.

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providing prompt and effective control of infectivity once the fungus has established itself in tissues or spread to internal organs. Therefore, host resistance to fungi appears to be dependent on the induction of cellular immunity, mediated by T lymphocytes, cytokines and a number of effector phagocytes.

**Th1/Th2 cells**

Generation of a dominant Th1 response driven by IL-12 is essentially required for the expression of protective immunity to fungi. Through the production of the signature cytokine IFN-γ and help for opsonizing antibodies, the activation of Th1 cells is instrumental in the optimal activation of phagocytes at sites of infection. Therefore, the failure to deliver activating signals to effector phagocytes may predispose patients to overwhelming infections, limit the therapeutic efficacy of antifungals and antibodies and favor persistency and/or commensalism [26]. Immunological studies in patients with polar forms of paracoccidioidomycosis demonstrate an association between Th1-biased reactivity and the asymptomatic and mild forms of the infection, as opposed to the positive correlation of Th2 responses with the severity of the disease. Not surprisingly, therefore, patients with disseminated or relapsing infection show defective production of IFN-γ and DTH anergy, associated with elevated levels of type 2 cytokines (IL-4 and IL-5), IgE, IgG4 and IgA and eosinophilia, which are markers of poor prognosis in endemic mycoses [26]. As already mentioned, patients with inborn errors of the IL-12/IL-23/IFNγ-mediated immunity are susceptible to disseminated paracoccidioidomycosis [36,37].

IL-4 acts as the most potent proximal signal for commitment to Th2 reactivity that dampens protective Th1 responses and favors fungal allergy. IL-4 may both deactivate and activate phagocytes and DCs for certain specialized function; for instance, it may inhibit the antifungal effector activities of phagocytes, yet may promote IL-12 production by DCs [92]. Thus, the most important mechanism underlying the inhibitory activity of IL-4 in infections relies on its ability to act as the most potent proximal signal for commitment to Th2 reactivity that dampens protective Th1 responses and favors fungal allergy. In atopic subjects and neonates, the suppressed DTH response to fungi is associated with elevated levels of antifungal IgE, IgA, and IgG. However, susceptibility to fungal infections may not always be associated with an overt production of IL-4 [6].

**The inflammatory Th17 pathway**

IL-12, by initiating and maintaining Th1 responses, was thought to be responsible for overreacting immune and autoimmune disorders. This was also the case in fungal infections where immunoregulation proved to be essential in regulating inflammation and uncontrolled Th1/Th2 antifungal reactivity [10]. Over the past several years, the demise of a Th1/Th2 dichotomy paradigm has been accompanied by a renaissance in probing the basic tenets of CD4+ T cell biology. As a result, instead of only two distinct ‘fates’ for developing T cells, research has identified alternative fates and more flexibility in T cell cytokine production than previously envisioned.

Th17 cells are now thought to be a separate lineage of effector Th cells contributing to immune pathogenesis previously attributed to the Th1 lineage [93]. Naïve mouse CD4+ T cells activated in the presence of TGF-β and IL-2 upregulate expression of the transcription factor Foxp3 (forkhead box P3) and develop into ‘inducible’ Tregs which suppress immune responses. In contrast, CD4+ T cells cultured with TGF-β and IL-6 express the transcription factor RORγt (retinoid-related orphan receptor gamma t) and become Th17 cells that are stabilized by DC-derived IL-23 [94,95].

IL-12 and IL-23 are members of a family of proinflammatory heterodimeric cytokines that share a common p40 subunit linked to the IL-12p35 chain or the IL-23p19 chain [96]. Th17 cells, which produce IL-17 preferentially, promote neutrophil-mediated inflammation and, although linked to the resistance to several bacterial and parasitic infections, correlate with disease severity and immunopathology in diverse infections [97]. With growing understanding of the contribution of the IL-23/Th17 axis to various organ-related autoimmune and inflammatory diseases [98], there has been an interest in targeting many aspects of this pathway for therapeutic interventions.

Recent results showed that Th17 cells are induced in fungal infections, through TLR- and non-TLR-dependent signalling [38,39,77,99,100]. Although IL-17 contributed to neutrophil mobilization in disseminated candidiasis [101] and participated in host defense to *P. carinii* [102], IL-17-producing T cells have also been described in the local granulomatous response to *H. capsulatum* [99], a finding suggesting that IL-17 could be a key element of pyogranulomatous and granulomatous host response to fungi. The Th17 pathway – and not the uncontrolled Th1 response – that is associated with defective pathogen clearance, failure to resolve inflammation and to initiate...
protective immune responses to *Candida* and *Aspergillus* (Fig. 2). Both IL-23 and IL-17 impaired the antifungal effector activities of neutrophils even in the presence of IFN-γ, a finding suggesting that the Th17 effector pathway prevails over the Th1 pathway. As a matter of fact, IL-12 and IL-23 cross-regulated each other and therefore, the relative levels of these cytokines may be the key to the outcome of infection. In addition, both cytokines activated the inflammatory program of neutrophils by counteracting the IFN-γ-dependent activation of indoleamine 2,3-dioxygenase (IDO), known to limit the inflammatory status of neutrophils against fungi [103,104] (see below), as well as by inducing the release of metalloproteinases and oxidants, which likely accounts for the high inflammatory pathology and tissue destruction associated with Th17 cell activation. The above findings, together with the ability of both IL-17 and IL-23 to reduce apoptosis of neutrophils, are thus consistent with the results obtained in IL-17 receptor-deficient mice with disseminated candidiasis [101]. It is of interest that induction of arginase 1, a metabolic pathway associated with IL-23 [105], is one virulence mechanism of *C. immitis* [106].

The finding that IL-23 and IL-17 promote inflammation while subverting protective antifungal immunity may serve to accommodate the paradoxical association of chronic inflammatory responses with intractable forms of fungal infections where fungal persistence occurs in the face of an ongoing inflammation. More generally, the Th17 pathway could be involved in the immunopathogenesis of chronic fungal diseases where persistent fungal antigens may maintain immunological dysreactivity. In this regard, Th17 activation could account for the exacerbation of autoimmunity induced by fungal β-glucans [107], despite the fact that zymosan containing β-glucans promotes immunological tolerance [108]. This confirms the multiplicity of receptor pathways contributing to Th17 activation (Fig. 1). Interestingly, the finding that Th17 cell activation occurs through the TLR/MyD88 signaling pathway suggests that conditions of high-threat inflammation may represent a local environmental factor that predispose to Th17 activation in fungal infections. In this scenario, the unrestricted fungal growth will result from the activation of not only pathogenic Th17 cells but also non protective Th2 cells, whose activation is strictly dependent on fungal burden.

These new findings provide a molecular connection between the failure to resolve inflammation and lack of antifungal immune resistance and point to strategies for immune therapy of fungal infections that attempt to limit inflammation to stimulate an effective immune response. As a matter of fact, IL-17 neutralization increased fungal clearance, ameliorated inflammatory pathology and restored protective Th1 antifungal resistance [11]. The finding that IL-17 neutralization greatly ameliorates pulmonary aspergillosis in a mouse model of CGD [109], further points to the therapeutic utility of immunomodulatory strategies aimed at reducing Th17-driven hyper-inflammation in fungal infections (see below).

Collectively, these new findings may help to accommodate fungi, either commensals or ubiquitous, within the immune homeostasis and its dysregulation. If the
ability to subvert the inflammatory program through the activation of the IL-23/IL-17 axis may eventually lead to immune dysregulation, their ability to activate Tregs (see below), that are integral and essential

compensate for the protective role of Tregs. As already discussed, a reciprocal relationship has been described between the development of Th17 cells and that of Tregs is prevented. However, despite the excitement raised by the new findings much remains to be learned, including the relationships among the various cytokine-producing T cell subsets, in mice and men [110]. For instance, the finding that mouse CD4+CD25+FoxP3+ cells can be ‘converted’ in vitro into IL-17-secreting T helper cells [111], indicates more flexibility in cytokine production than would be predicted from the terminal differentiation model of Th1/Th2 cells. Ultimately, the potential ‘interconvertability’ of Th17 subsets is compounded in humans by the well known plasticity of human CD4+ T cell differentiation [110]. Future studies will highlight the relative contribution of the various populations of IL-17-producing cells that have been recently described [112] to the pathogenesis of infections and diseases caused by the different fungi.

Dampening inflammation and allergy to fungi: the role of Tregs

To limit the pathologic consequences of an excessive inflammatory cell-mediated reaction, the immune system resorts to a number of protective mechanisms. CD4+ T cells making immunoregulatory cytokines such as IL-10, TGF-β and IL-4 have long been known and discussed in terms of immune deviation or class regulation [113]. Immune responses to fungi are modulated by one or more types of cells that perform a regulatory function. Tregs with tolerogenic activity have been described in fungal infections of both mice [104,114–116] and humans [117]. After over a decade of eclipse, suppressor T cells have regained a reputation as key controllers of antifungal immunity. Some cells with this function, such as CD4+ Foxp3+ natural regulatory T cells (nTregs), originate in the thymus and pre-exist prior to infections whereas others may be induced as a consequence of infection (iTregs) or in conditions of impaired costimulatory signalling and in the presence of deactivating cytokines and drugs. Recent studies indicate that the intestine is a site of Foxp3+ iTreg development and that specialized intestinal DCs promote Foxp3 expression via a mechanism that is dependent on local TGF-β and retinoic acid, a vitamin A metabolite [118]. As already discussed, a reciprocal relationship has been described between the development of Foxp3+ Tregs and effector Th17 cells, so that naïve T cell activation in the presence of innate stimuli divert iTreg generation to Th17 generation [95,97].

A number of clinical observations suggest an inverse relationship between IFN-γ and IL-10 production in patients with fungal infections. High levels of IL-10, negatively affecting IFN-γ production, are detected in chronic candidal diseases, in the severe form of endemic mycoses and in neutropenic patients with aspergillosis [10]. However, although mannan and fungal polysaccharides negatively modulate CMI through the production of IL-10, solid evidence demonstrating a causal role for IL-10 in susceptibility to fungal infections is lacking. Recently, it has been suggested that, rather than causing the infection, IL-10 production may be a consequence of the infection [10]. This would predict that, in the case of chronic fungal infections, characterized by a state of chronic inflammation, IL-10 could be the homeostatic host-driven response aimed at keeping, however possible, inflammation under control.

With pathogens like fungi that have a complex pathogenesis, multiple types of regulatory cells could influence the outcome (Fig. 3). A fine balance is established between Treg responses, effector components of immunity and the pathogen [10]. The consequence of Treg activity is less damage to the host but also fungal persistence. However, the Treg responses may handicap the efficacy of protective immunity [119]. In mice with candidiasis, CD4+CD25+ Tregs, producing IL-10 and TGF-β, prevented sterilization of the fungus from the gastrointestinal tract and allow fungal persistence and the occurrence of memory immunity [116]. Fungal growth, inflammatory immunity, and tolerance to the fungus were all controlled by the coordinate activation of nTregs – limiting early inflammation at the sites of infection – and pathogen-specific iTregs, which regulated the expression of adaptive Th immunity in secondary lymphoid organs. nTreg cells required the Toll/IL-1 receptor (TIR)-domain-containing adaptor protein inducing IFN-β (TRIF) pathway for migration to inflamed sites, where the MyD88 pathway would then restrain their suppressive function. Subsequent inflammatory TH1-type immunity (TRIF) was modulated by iTreg cells, which required pathway as well, and which acted through activation of IDO in DCs and Th17 cell antagonism. Thus an optimally protective reactivity balanced between immunity and tolerance to the fungus requires sequential inputs from the TRIF and the Drosophila myeloid differentiation primary response gene 88 (MyD88) pathways, as well as tryptophan catabolism as a long-term regulatory system [38].

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Because, both the recovery of the fungus from the gastrointestinal tract and the detection of underlying Th1 reactivity, such as DTH and lymphoproliferation, can fluctuate in healthy subjects, it is likely that Tregs mediate tolerance to the fungus at the site of colonization. It has long been presumed that the ability of *C. albicans* to persist in host tissue might involve primarily the immunosuppressive property of cell wall glycoproteins. CMC, although encompassing a variety of clinical entities [6] has been associated with autoimmune polyendocrinopathy-candidiasis-ectodermal dysplasia a condition in which Treg induction is defective [120]. As a matter of fact, in CMC the defective type 1 cytokine production does not concomitantly occur with the obvious increase of type 2 cytokine production (namely IL-4 or IL-5) but, more often, with IL-10 [6]. This finding suggests that an inherent alteration in receptor-mediated signaling in response to fungal polysaccharide may eventually predispose patients with CMC to a dysfunctional induction of Tregs. Whether and how the Th17 pathway fits within this scenario is presently unknown.

**Crosstalk between Treg subsets in infections**

Although the distinction between nTregs and iTregs in vivo is not always clear, particularly in highly inflammatory settings [118], the above-mentioned data indicate that fungal growth, inflammatory immunity, and tolerance to fungi, in the respiratory or the gastrointestinal mucosa, may be controlled by the coordinate activation of nTregs – limiting early inflammation at the sites of infection – and pathogen-induced iTregs, which regulated the expression of adaptive Th immunity in secondary lymphoid organs (Fig. 3). Thus, different Treg cell populations may have the capacity to influence the emergence or function of one another. This was best illustrated in murine aspergillosis where, early in infection, inflammation was controlled by the expansion, activation and local recruitment of nTregs suppressing neutrophils through the combined actions of IL-10 and cytotoxic T lymphocyte antigen-4 (CTLA-4)– and IL-10-dependent mechanisms. Late in infection, and similarly in allergy, tolerogenic iTregs inhibited Th2 cells and prevent allergy to the fungus [104]. The level of inflammation and IFN-γ in the early stage set the subsequent adaptive stage by conditioning the IDO-dependent tolerogenic program of DCs and the subsequent activation and expansion of tolerogenic Tregs preventing allergy to the fungus. Therefore, regulatory mechanisms operating in the control of inflammation and allergy to the fungus are different but interdependent as the level of the inflammatory response early in infection may impact on susceptibility to allergy, in conditions of continuous exposure to the fungus.
nTregs, by affecting IFN-γ-production, indirectly exert a fine control over the induction of late tolerogenic iTregs. Thus, a unifying mechanism linking natural Treg cells to tolerogenic regulatory Treg cells in response to the fungus is consistent with the revisited ‘hygiene hypothesis’ of allergy in infections – that is, an early reduction in microbial burden may predispose to allergy, and may provide at the same time mechanistic explanations for the significance of the variable level of IFN-γ seen in allergic diseases and asthma and for the paradoxical worsening effect on allergy of Th1 cells.

Collectively, these observations suggest that the capacity of Tregs to inhibit aspects of innate and adaptive immunity is pivotal in their regulatory function and further support the concept of ‘protective tolerance’ to fungi. implying that a host’s immune defense may be adequate for protection without necessarily eliminating fungal pathogens – which would impair immune memory – or causing an unacceptable level of tissue damage [10].

**Immunity or tolerance: the decisive role of the tryptophan metabolic pathway**

The inflammatory/anti-inflammatory state of DCs is strictly controlled by the kynurenine pathway in tryptophan catabolism and involves IDO [121]. IDO has a complex role in immunoregulation in infection, pregnancy, autoimmunity, transplantation, and neoplasia [122]. IDO-expressing DCs are regarded as regulatory DCs specialized to cause antigen-specific deletional tolerance or induction of CD4⁺CD25⁺ Tregs [123]. These findings establish a mutual interaction between DCs and Tregs for the upkeep of immunological tolerance. In experimental fungal infections IDO blockade greatly exacerbated infections, the associated inflammatory pathology and swept away resistance to reinfection, as a result of deregulated innate and adaptive immune responses caused by the impaired activation and functioning of suppressor CD4⁺CD25⁺ Tregs producing IL-10 [10]. More recently, while capable of inducing the Foxp3-encoding gene transcriptionally, tryptophan catabolites were also found to suppress the gene encoding RORγt, the Th17 lineage specification factor [38]. Thus regulation of iTregs/Th17 balance at the host/pathogen further emphasizes the pivotal role of tryptophan catabolism and IDO in tolerogenesis and prevention of inflammation and allergy [11]. As a matter of fact, similar to IL-17 neutralization, administration of exogenous kynurenines restricted the activation of IL-17-producing T cells, decreased inflammation and cured CGD mice from pulmonary aspergillosis [109].

The IDO mechanism has revealed an unexpected potential in the control of inflammation not only in infection but also in airway allergy, a conditions in which tolerogenic DCs could have a protective function [124]. IDO expression is paradoxically up-regulated in patients with allergy or autoimmune inflammation, a finding suggesting the occurrence of a homeostatic mechanism to halt ongoing inflammation [11]. As already discussed, a unifying mechanism linking nTregs to tolerogenic iTregs via IDO appears to be at work in murine allergic aspergillosis.

Recent data have confirmed the protective role of the IDO/Tregs axis in fungal allergy [125]. In this model, modulation of tryptophan catabolism via the glucocorticoid-induced tumor necrosis factor receptor (GITR) and its ligand, GITRL, inhibited Th2-cell responses and allergy and induced the expression of Foxp3⁺ Tregs through mechanisms dependent on IDO induction by components of the noncanonical NF-κB signaling pathway [125]. Thus, induction of IDO could be an important mechanism underlying the anti-inflammatory action of corticosteroids [125].

The implication for IDO in immunoregulation in fungal infections has several important implications. As C. albicans is a commensal of the human gastrointestinal and genitourinary tracts and IFN-γ is an important mediator of protective immunity to the fungus, the IFN-γ/IDO axis may accommodate fungal persistence in a host environment rich in IFN-γ. In its ability to induce Th1 immunity within a regulatory environment and to prevent Th17 development, IDO expression may correlate with the occurrence of local tolerogenic responses. Alternatively, the high levels of IL-10 production may be a consequence of IDO activation by the fungus, impairing antifungal Th1 immunity and thus favoring persistent infection. The fact that hyphae, more than yeasts, activate the expression of IDO [38], further suggests that differential sensing of fungal morphotypes through distinct recognition receptors may promote distinct immune responses and that fungal hyphae, more than yeasts, may promote tolerance and thus contribute to commensalism and eventually to immunoevasion. Because germinating *Aspergillus* conidia promote inflammatory responses by subverting tolerance [104], the central role of tolerance at the fungus/host interface and the pivotal role of tryptophan catabolism in the tolerant state are both emphasized. Ultimately, the manipulation of Tregs by fungi gives further support to the notion that gut microbiota may function as a major regulator of immunological tolerance both locally and at distant sites [126].
Conclusions: the anti-inflammatory strategy has come of age

A finely orchestrated balance between activating and inhibitory signals is fundamental for the ability of the immune system to effectively attack and eliminate pathogenic fungi and/or coexist with commensals without reacting against self-antigens. Derangements of this balance may underlie the pathogenesis of chronic infections and autoimmune inflammatory diseases. The new discoveries in the field of fungal immunology have offered new grounds for a better comprehension of cells and immune pathways that are amenable to manipulation in patients with or at risk of fungal infections (Fig. 4). Preclinical studies have shown the potential therapeutic role of a variety of cytokines, growth factors and immunomodulators in fungal infections [127,128]. The Th1/Th2 balance itself can be the target of immunotherapy [129]. However, the new findings provide the basis for novel immunomodulatory therapies that are strictly required to limit inflammation in order to stimulate an effective immune response. Notwithstanding the redundancy and overlapping repertoire of antifungal effector mechanisms, the pivotal role of different types of Tregs in the control of Th1/Th2 inflammatory responses as well as in Th17 antagonism, suggests that manipulation of Tregs could be a promising therapeutic approach devoid of risks associated with interference with homeostatic mechanisms of the immune system. In this regard, the potential of the anti-TNF antibody infliximab to induce anti-inflammatory cytokines IL-10 or TGF-β via retrograde signalling or through induction of a certain subset of Tregs is of interest [130]. Likewise, tryptophan metabolites and Th17 inhibitors [11] are likely candidate as potent regulators capable of taming overzealous or heightened inflammatory host responses to the benefit of pathogen control and host survival at the pathogen/host interface that is a reciprocal two-edged sword interaction.

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Conflict of interest

The author declares that no conflicts of interest exist.

References


83 Lindell DM, Ballinger MN, McDonald RA, Toews GB, Huffnagle GB. Immunologic homeostasis during infection: coexistence of strong pulmonary cell-mediated immunity to secondary Cryptococcus neoformans infection while the primary infection still persists at low levels in the lungs. *J Immunol* 2006; 177: 4652–4661.


112 Jankovic D, Trinchieri G. IL-10 or not IL-10: that is the question. *Nat Immunol* 2007; 8: 1281–1283.


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